Interagency Metabolic Engineering Program

Panel Manager - Dr. Fred Heineken, National Science Foundation
USDA Program Contacts - Mr. Jeff Conrad, Dr. Liang-Shiou Lin, and Dr. Gail McLean

The USDA, NRI participates in the Interagency Announcement of Opportunities in Metabolic Engineering (ME). This Announcement is the result of a collaborative effort among the Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Environmental Protection Agency, National Aeronautics and Space Administration, National Institute of General Medical Sciences (National Institutes of Health), and the National Science Foundation. The intent of this announcement is to provide an opportunity for an inter-agency granting activity in ME and to draw attention to Federal research and development interests in this area.

For the purposes of the announcement, metabolic engineering is defined as an approach to the understanding and utilization of metabolic processes. It implies the targeted and purposeful alteration of metabolic pathways found in an organism in order to better understand and utilize cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. Topics of particular interest are:
- Instrumentation, sensors, new analytical tools, and new experimental methods which facilitate the study of metabolic pathways, especially those technologies that allow the examination of individual cells.
- Quantitative and conceptual models integrated with experimental studies that better characterize the regulation and integration of complex, interacting metabolic pathways.
-The use of bioinformatics to deduce the structure, function, and regulation of major metabolic pathways.

The projects listed below are those projects for which the USDA, NRI provided research support during FY 2000.

2000-02778 Maximizing Ethanol Production by Engineered Pentose-Fermenting *Zymomonas mobilis*

Kompala, D.S.
University of Colorado, Boulder; Department of Chemical Engineering; Boulder, CO 80309-0424
Grant 99-35505-8685; $300,000; 3 Years

This research seeks to initiate a multi-disciplinary collaboration to maximize ethanol production by metabolically engineered *Zymomonas mobilis* strains from mixtures of hexose and pentose sugars through the application of metabolic flux control techniques. A collaborative research is proposed between the Principal Investigator, a biochemical engineer skilled in the mathematical modeling of recombinant microorganisms at the University of Colorado, Boulder and two Collaborating Investigators at the Biotechnology Branch of the National Renewable Energy Laboratory (NREL) in Golden, Colorado.

The scientists at NREL have successfully engineered the ethanol fermenting *Zymomonas mobilis* to broaden its substrate utilization range from only the hexoses, glucose and fructose, to include the additional pentose sugars, xylose and arabinose, found in renewable biomass feedstocks and agricultural residues. The pentose

fermentation was accomplished by introducing two genes encoding for key enzymes in the xylose assimilation pathway, three genes encoding for key enzymes in the arabinose assimilation pathway and two genes encoding for key enzymes in the pentose phosphate pathway into the efficient ethanol producer *Z. mobilis*.

In the proposed research, detailed kinetic models of the integrated pathways will be developed to identify potential sites for enhancing the overall ethanol metabolic flux. Intracellular metabolic intermediates will be measured using powerful non-invasive, real-time analytical tools to identify bottlenecks and investigate the predictions from our kinetic model simulations. New genetic engineering tools will be developed in *Z. mobilis*, and improved strains will be constructed for efficient ethanol fermentation from hexose and pentose sugar mixtures, found in biomass and agricultural residues.

2000-03654 Metabolic Engineering to Study the Regulation/Plasticity of and to Modify Diterpene Metabolism in Trichome Gland Cells

Wagner, G. J.
University of Kentucky; Department of Agronomy; Lexington, KY 40546
Grant 99-35321-8687; $100,001; 3 Years

We propose to determine the plasticity of terpene metabolism in trichome glands in order to understand metabolic regulation in this specialized plant tissue. Understanding of metabolic regulation and plasticity is needed if we are to exploit (using metabolic engineering) this tissue to produce useful biochemicals (molecular farming). Trichome glands of certain plants represent a potential high-capacity factory for producing certain chemicals for commerce. Metabolic regulation in this tissue must be understood if this system is to be exploited.